

Fig. 1. Schematic representation of the steps involved in the isolation of retinoid-regulated genes using the differential display technique. The cloned products isolated in step 6 can then be used for sequencing, Northern blotting or screening of cDNA libraries. P1, P2 and P3 correspond to fragments from RA induced mRNAs. P4 represents a PCR product from an mRNA which is down-regulated.

FIG. 1

FIG. 2

A



C

MetGlyLeuTyrThrLeuMetValThrPheLeuCysThrIleValLeuProValLeuLeu 20  
PheLeuAlaAlaValLysLeuTrpGluMetLeuMetIleArgArgValAspProAsnCys 40  
ArgSerProLeuProProGlyThrMetGlyLeuProPheIleGlyGluThrLeuGlnLeu 60  
IleLeuGlnArgArgLysPheLeuArgMetLysArgGlnLysTyrGlyCysIleTyrLys 80  
ThrHisLeuPheGlyAsnProThrValArgValMetGlyAlaAspAsnValArgGlnIle 100  
LeuLeuGlyGluHisLysLeuValSerValGlnTrpProAlaSerValArgThrIleLeu 120  
GlySerAspThrLeuSerAsnValHisGlyValGlnHisLysAsnLysLysLysAlaIle 140  
MetArgAlaPheSerArgAspAlaLeuGluHisTyrIleProValIleGlnGlnGluVal 160  
LysSerAlaIleGlnGluTrpLeuGlnLysAspSerCysValLeuValTyrProGluMet 180  
LysLysLeuMetPheArgIleAlaMetArgIleLeuLeuGlyPheGluProGluGlnIle 200  
LysThrAspGluGlnGluLeuValGluAlaPheGluGluMetIleLysAsnLeuPheSer 220  
LeuProIleAspValProPheSerGlyLeuTyrArgGlyLeuArgAlaArgAsnPheIle 240  
HisSerLysIleGluGluAsnIleArgLysLysIleGlnAspAspAspAsnGluAsnGlu 260  
GlnLysTyrLysAspAlaLeuGlnLeuLeuIleGluAsnSerArgArgSerAspGluPro 280  
PheSerLeuGlnAlaMetLysGluAlaAlaThrGluLeuLeuPheGlyGlyHisGluThr 300  
ThrAlaSerThrAlaThrSerLeuValMetPheLeuGlyLeuAsnThrGluValValGln 320  
LysValArgGluGluValGlnGluLysValGluMetGlyMetTyrThrProGlyLysGly 340  
LeuSerMetGluLeuLeuAspGlnLeuLysTyrThrGlyCysValIleLysGluThrLeu 360  
ArgIleAsnProProValProGlyGlyPheArgValAlaLeuLysThrPheGluLeuAsn 380  
GlyTyrGlnIleProLysGlyTrpAsnValIleTyrSerIleCysAspThrHisAspVal 400  
AlaAspValPheProAsnLysGluGluPheGlnProGluArgPheMetSerLysGlyLeu 420  
GluAspGlySerArgPheAsnTyrIleProPheGlyGlyGlySerArgMetCysValGly 440  
LysGluPheAlaLysValLeuLeuLysIlePheLeuValGluLeuThrGlnHisCysAsn 460  
TrpIleLeuSerAsnGlyProProThrMetLysThrGlyProThrIleTyrProValAsp 480  
AsnLeuProThrLysPheThrSerTyrValArgAsn 492

B

→  
TGCCAGTGGACAATCTCCCTACCAAAATTCAGTATGATCCAGAAATTA 50  
GCCTAAACCGGAGCCTTTGTACATATGTTTTTATTTTAGATGAACGTGA 100  
TGTATTGGATATTTTCTAATTTGTTTATATAAAGCAGATGTTATATAAG 150  
TCTATGCGAAGAGCGAAAACGAGGCACTACTTCTCATGGATCACTGT 200  
AATGCTACAGAGTGTCTGTGATGATATTTTATAATGTAAGTTGTGTCATAT 250  
AGCTTTTGTACTGTATGCAACTTATTTAACTCGCTCTTATCTCATGGGT 300  
TTTATTTAATAAAACATGTTCTTACAAAAA  
←

D

P450RAI -8 -4 0 4 8  
ATCYP450 PFGGSRMCVKGKFAKVLK  
RATCYP4A1 \*\*\*\*\*P\*L\*P\*Y\*L\*R\*A\*S  
RABCYP4A5 \*\*S\*\*A\*N\*I\*\*Q\*\*MSEM\*  
CYP4503A12 \*\*S\*\*A\*N\*I\*\*Q\*\*MNE\*\*  
hCYTFAOH \*\*\*T\*P\*N\*I\*MR\*\*IMEM\*  
\*\*S\*\*\*\*N\*I\*\*Q\*\*MNE\*\*

005250 2848950

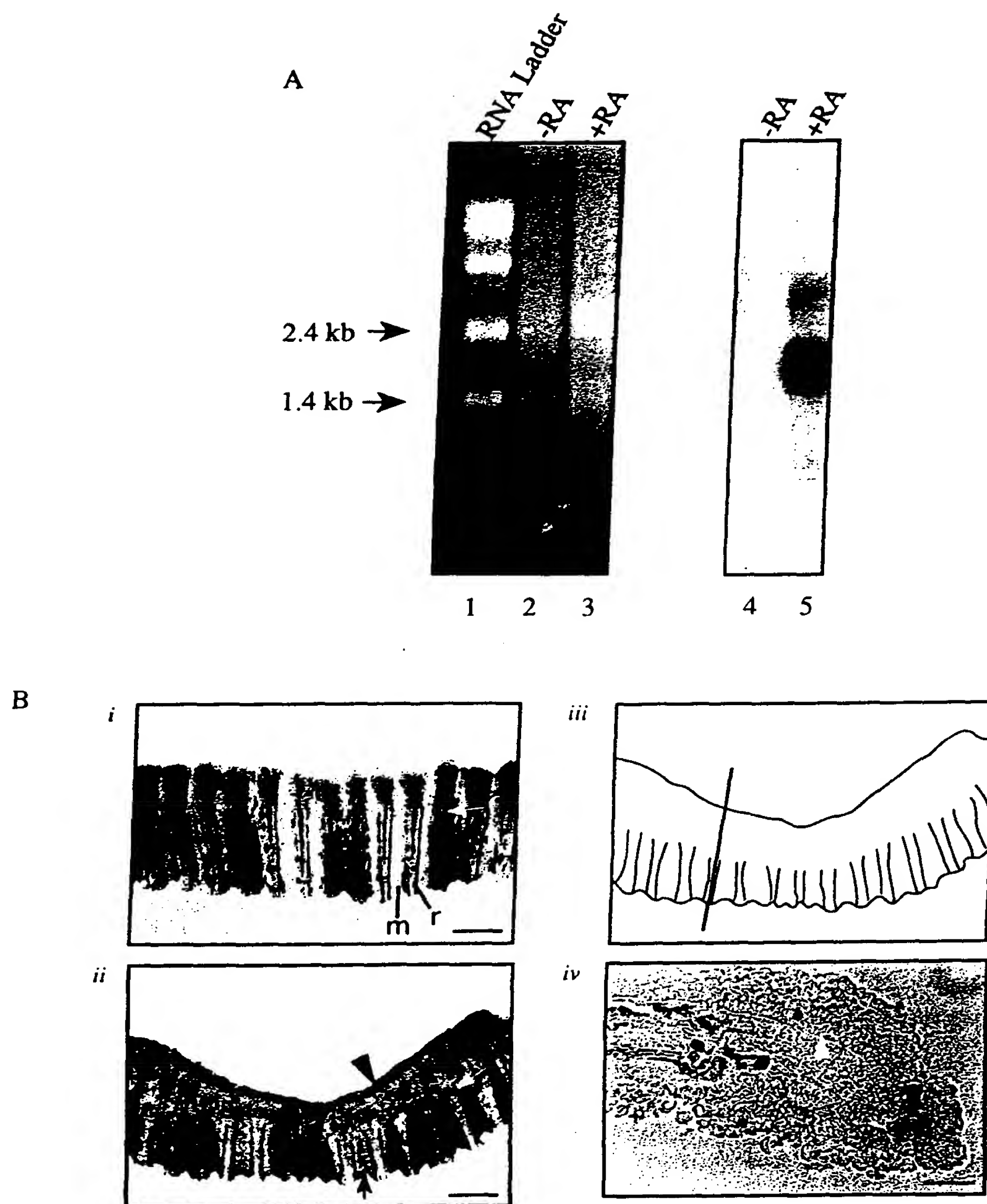
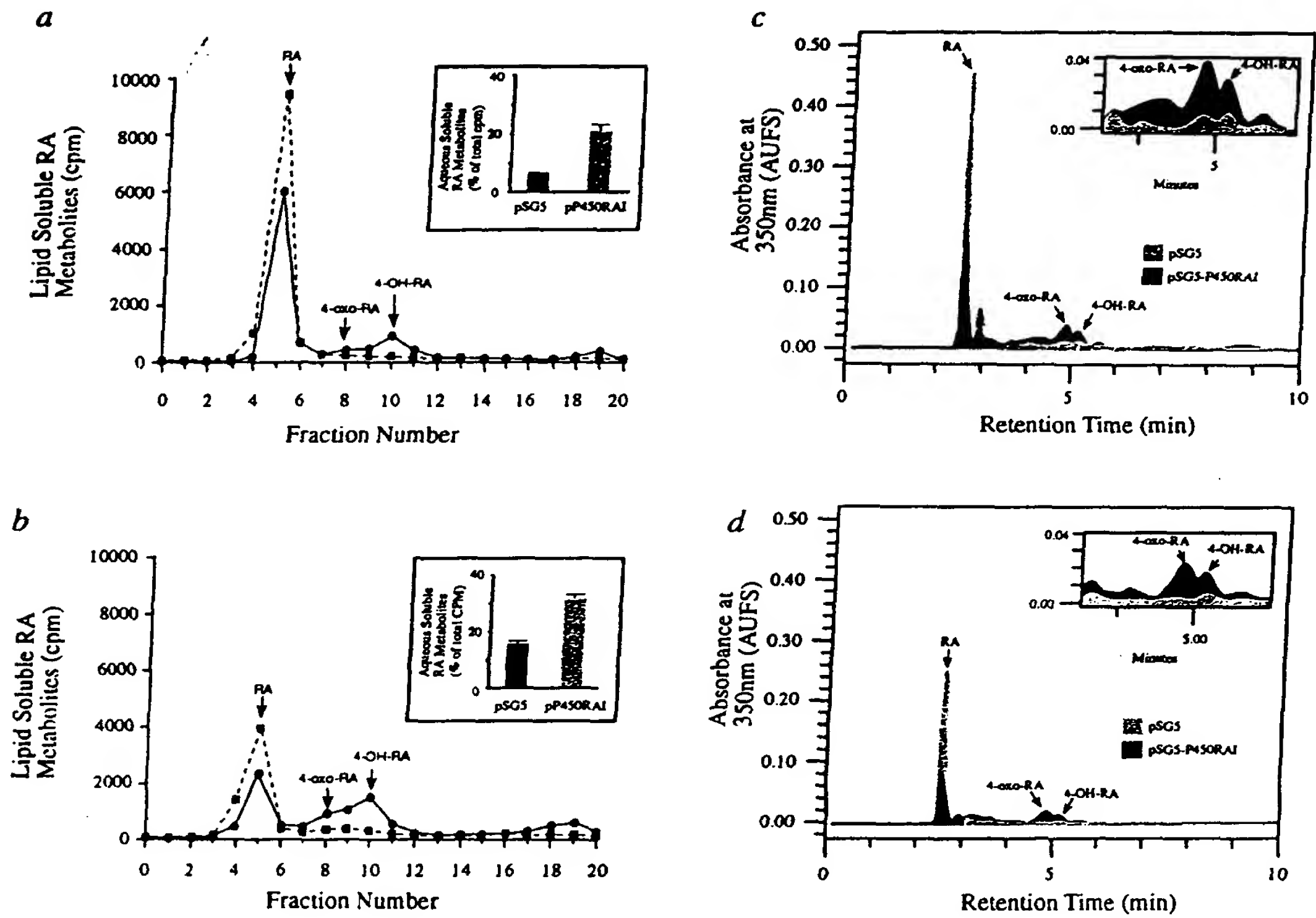


FIG. 3

FIG. 4



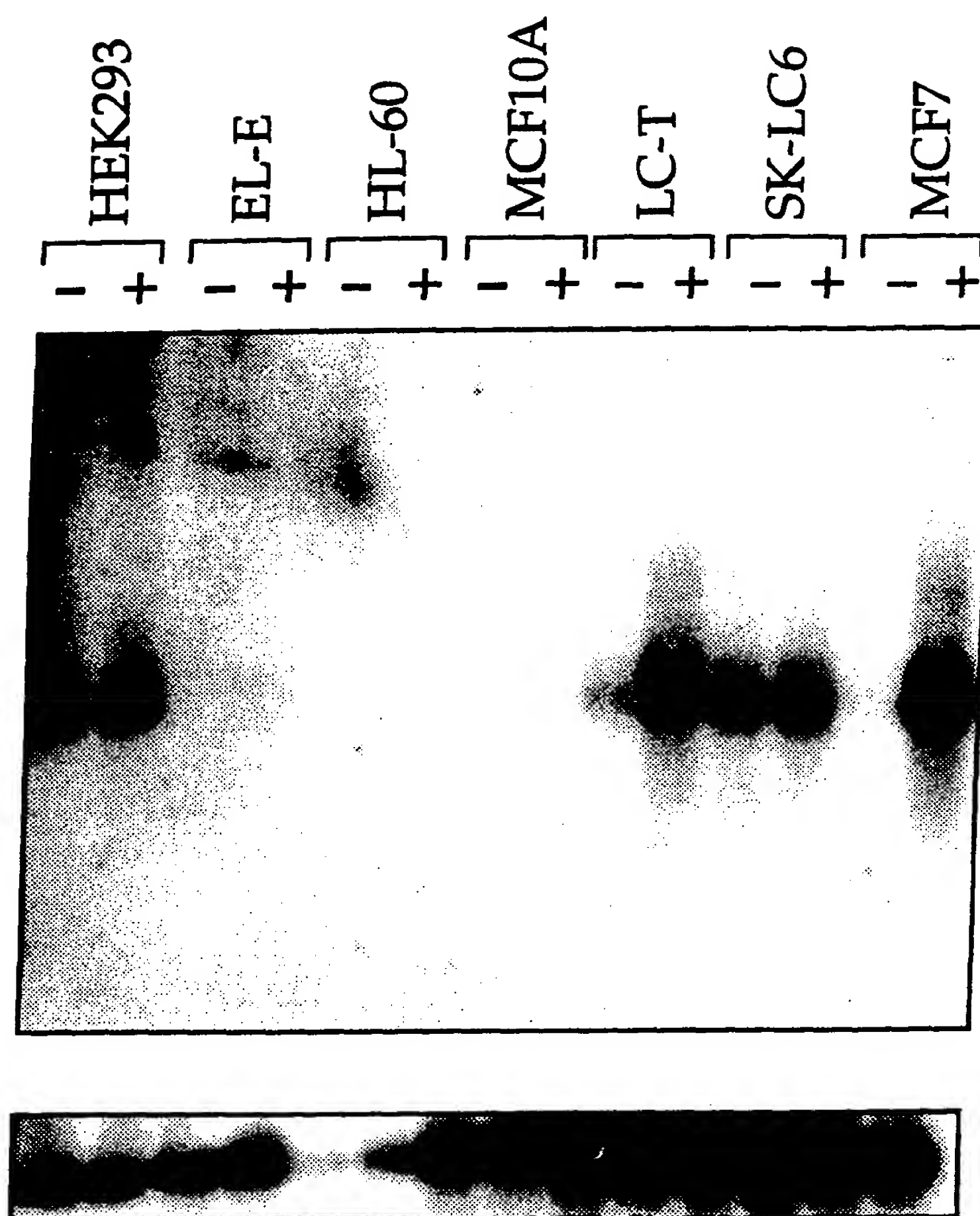


FIG. 5

005260 "2848960

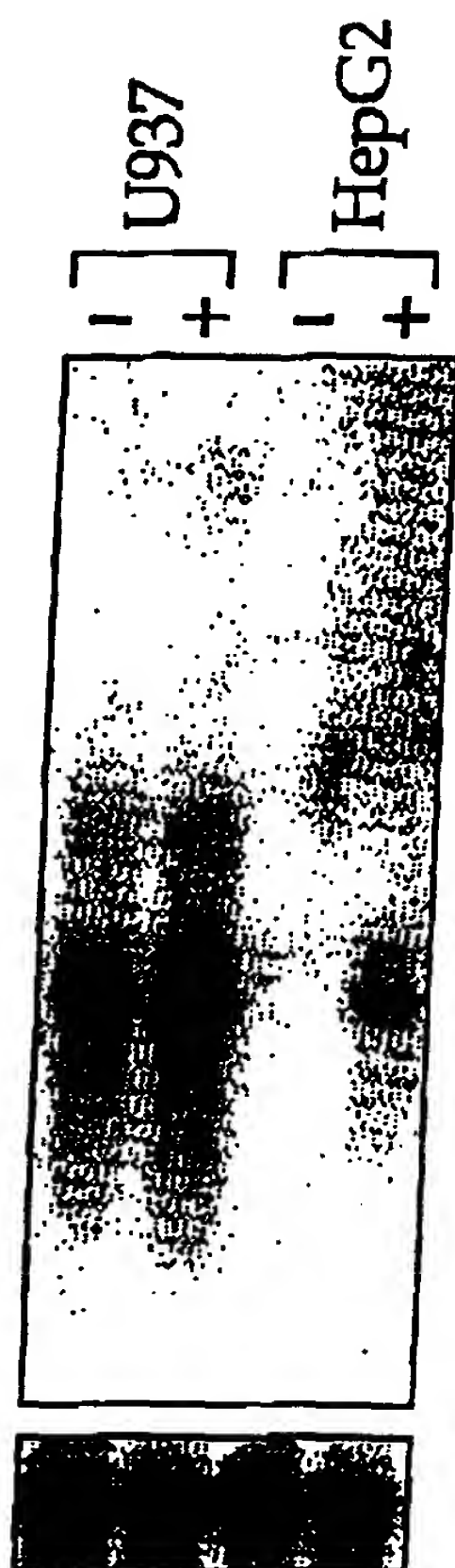


FIG. 6



009260 "28489960

NT2

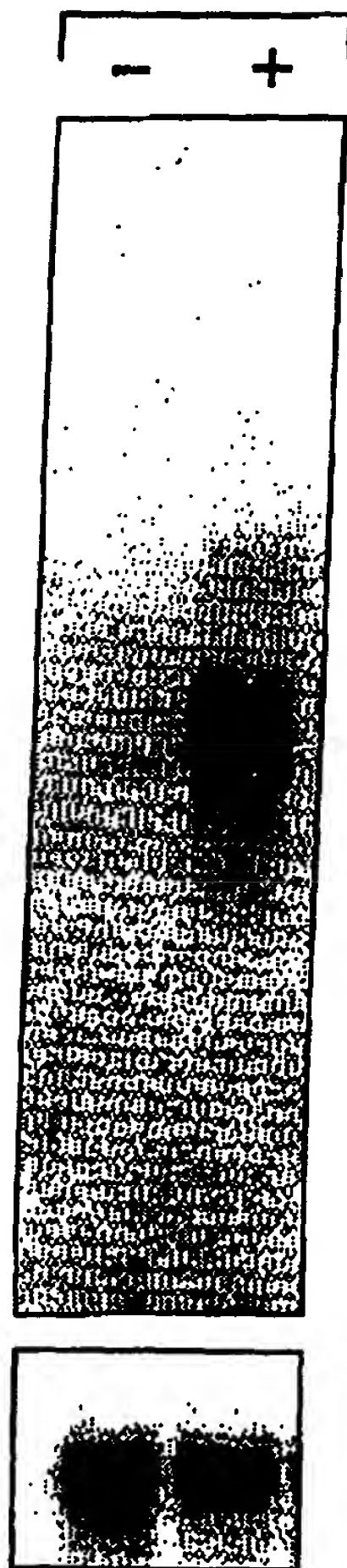


FIG. 7

005250 "28489950

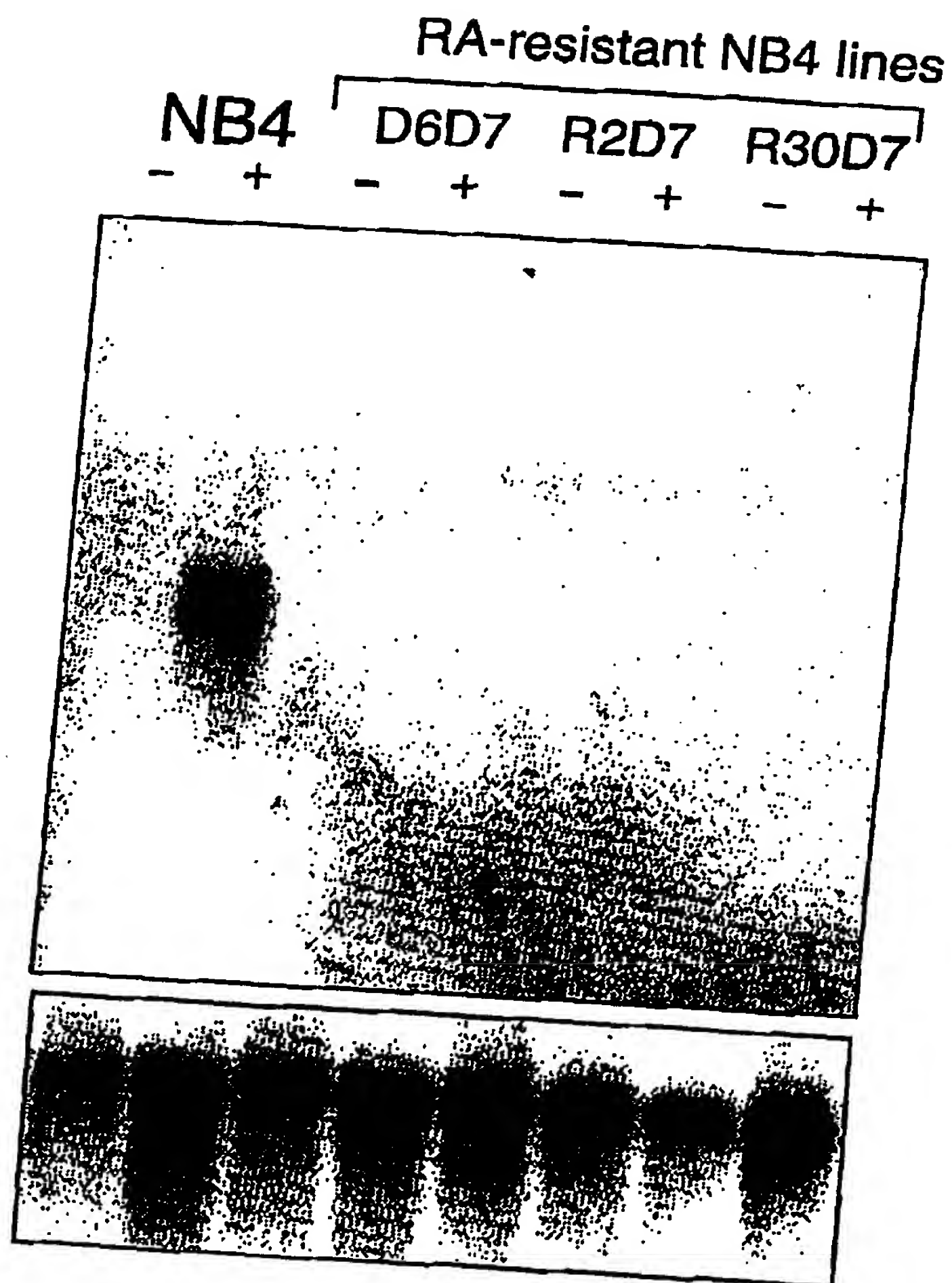


FIG. 8



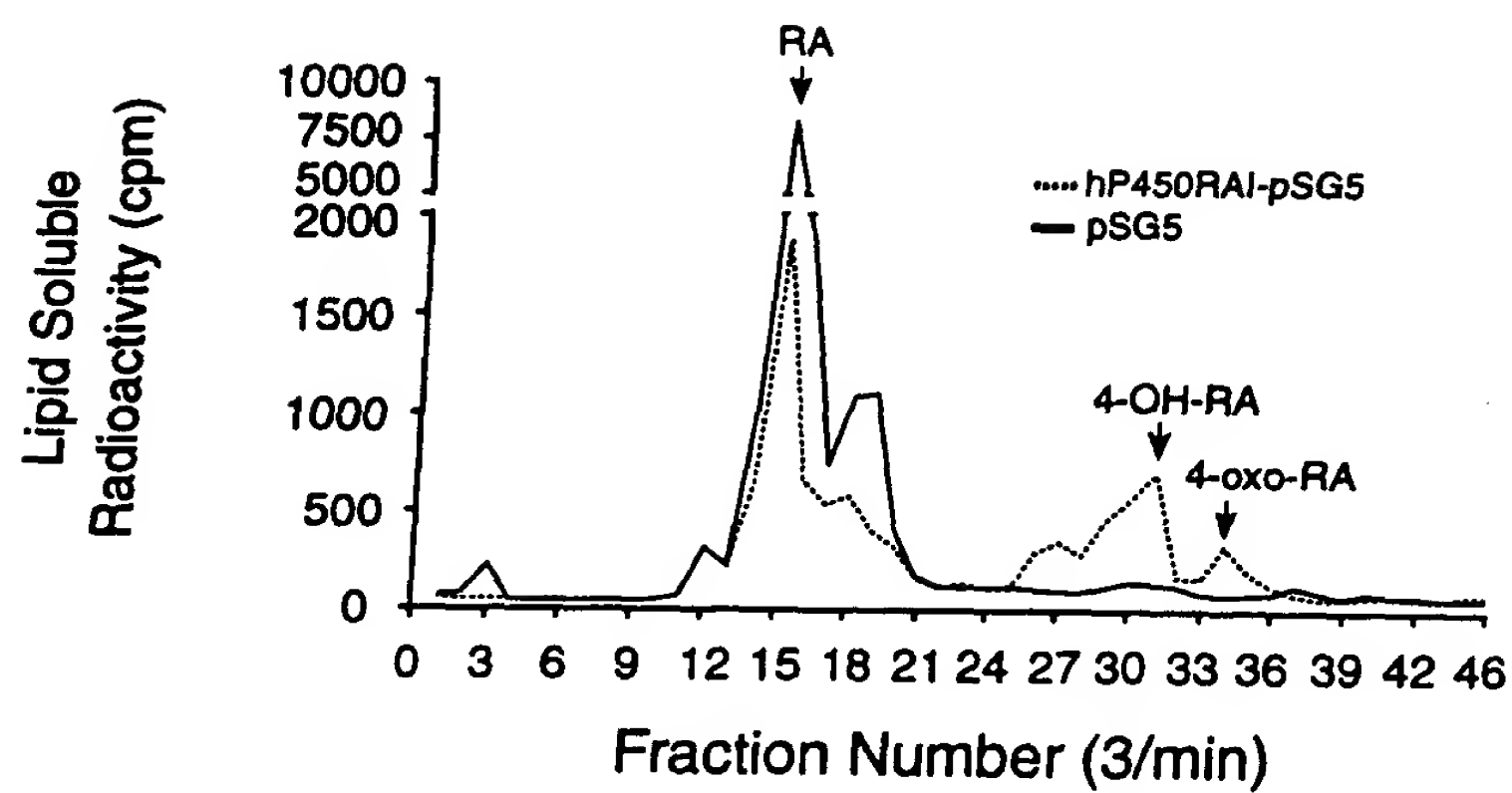
FIG. 9

mP450RAI	MGLPALLASALCTFVLPLLLFLAALKLWDLYCVSSRDRSCALPLPPGTMGFPFFGETLQM	60
hP450RAI	.....I.....G.....	60
zP450RAI	...YT.MVTF...I...V.....V...EMLMIRRV.PN.RS.....L..I.....L	60
mP450RAI	VLQRRKFLQMKRRKYGFIYKTHLFGRTVRVMGADNVRRILLGEHRLVSVHWPASVRTIL	120
hP450RAI	.....DD.....	120
zP450RAI	I.....R...Q...C.....N.....Q.....K...Q.....	120
mP450RAI	GAGCLSNLHDSSHKQRKKVIMQAFSREALQCYVLVIAEEVSSCLEQWLSCGERGLLVYPE	180
hP450RAI	.S.....R.....E...P..T...G.S.....	180
zP450RAI	.SDT...V.GVQ..NK..A..R....D..EH.IP..QQ..K.AIQE..Q-KDSCV.....	179
mP450RAI	VKRLMFRIAMRILLGCEPGPAGGGEDEQQLVFAFEEMTRNLFSLPIDVPFSGLYRGVKAR	240
hP450RAI	.....QL..D.DS.....M...	240
zP450RAI	M.K.....F..EQI--KT...E.....IK.....LR..	237
mP450RAI	NLIHARIEENIRAKIRRLQATEPDGGCKDALQLLIEHSWERGERLDMQALKQSSTELLFG	300
hP450RAI	.....Q.....CG.R.S.AGQ.....	300
zP450RAI	.F..SK.....K..QDDNENEQ-KY.....N.RRSD.PFSL..M.EAA.....	296
mP450RAI	GHETTASAATSLITYLGLYPHVLQKVREEIKSKGLLCKSNQDNKLDMETLEQLKYIGCVI	360
hP450RAI	.....L.....I.....	360
zP450RAI	.....T....VMF...NTE.V.....VQE.VEMGMYTPGKG.S..L.D....T....	356
mP450RAI	KETLRLNPPVPGGFRVALKTFELNGYQIPKGWNVYISICDTHDVADIFTNKEEFNPDRFI	420
hP450RAI	.....E.....S	420
zP450RAI	.....I.....V.P....Q.E..M	416
mP450RAI	VPHPEDASRFSFIPFGGGLRSCVGKEFAKILLKIFTVELARHCDWQLLNGPPTMKTSP TV	480
hP450RAI	A.....	480
zP450RAI	SKGL..G...NY.....S.M.....V.....L...TQ..N.I.S.....G..I	476
mP450RAI	YPVDNLPARFTYFQGDI	497
hP450RAI	.....H.H.E.	497
zP450RAI	.....TK..SYVRN-	492

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*a*



*b*

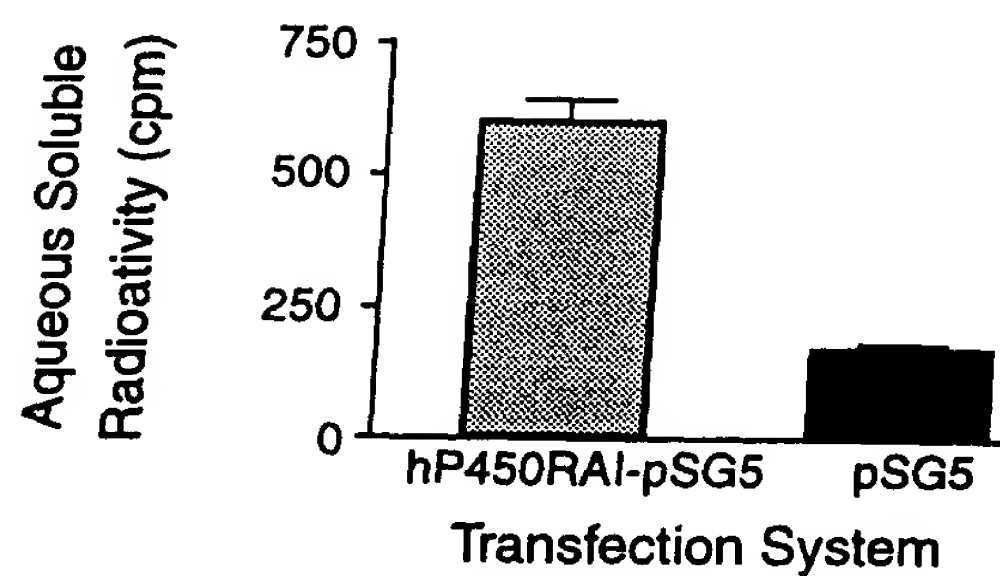
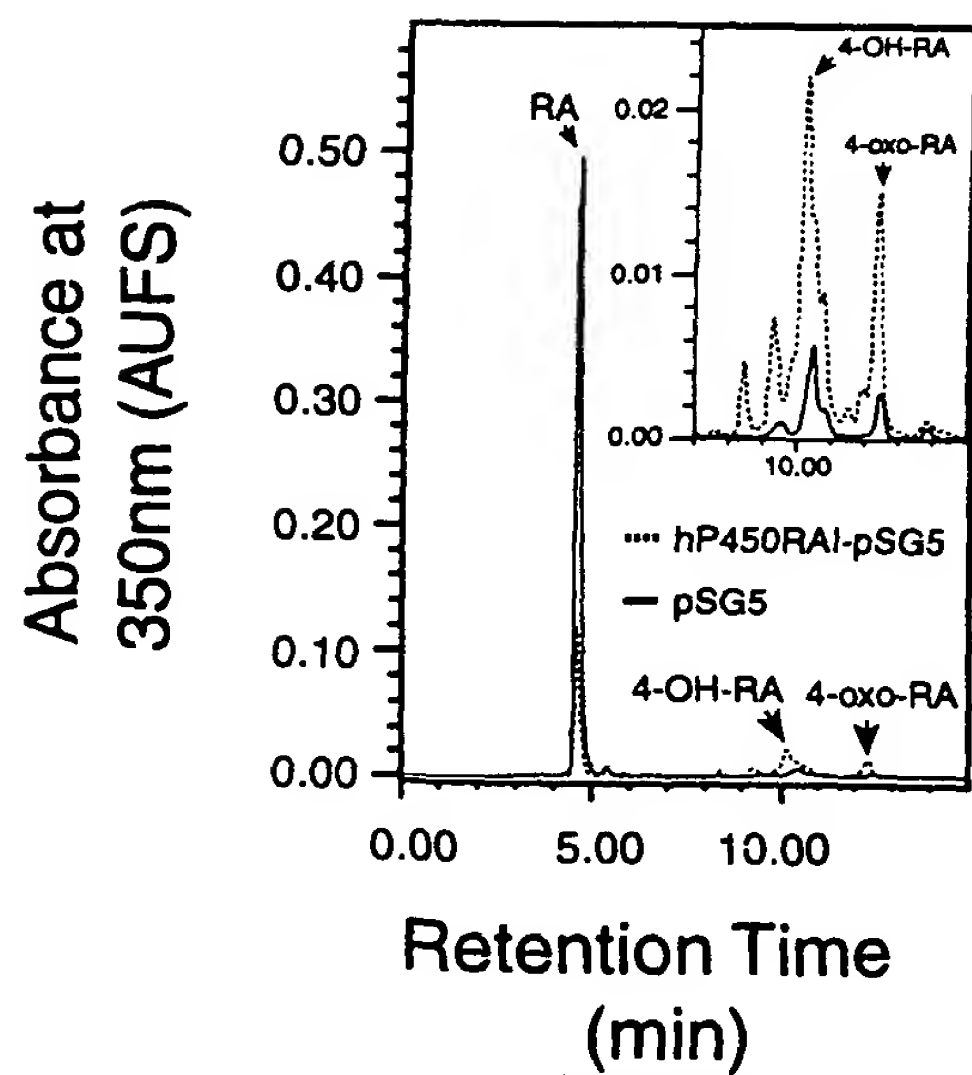


FIG. 10

*c*



4-oxo-RA Production in Transfected COS-1 Cells

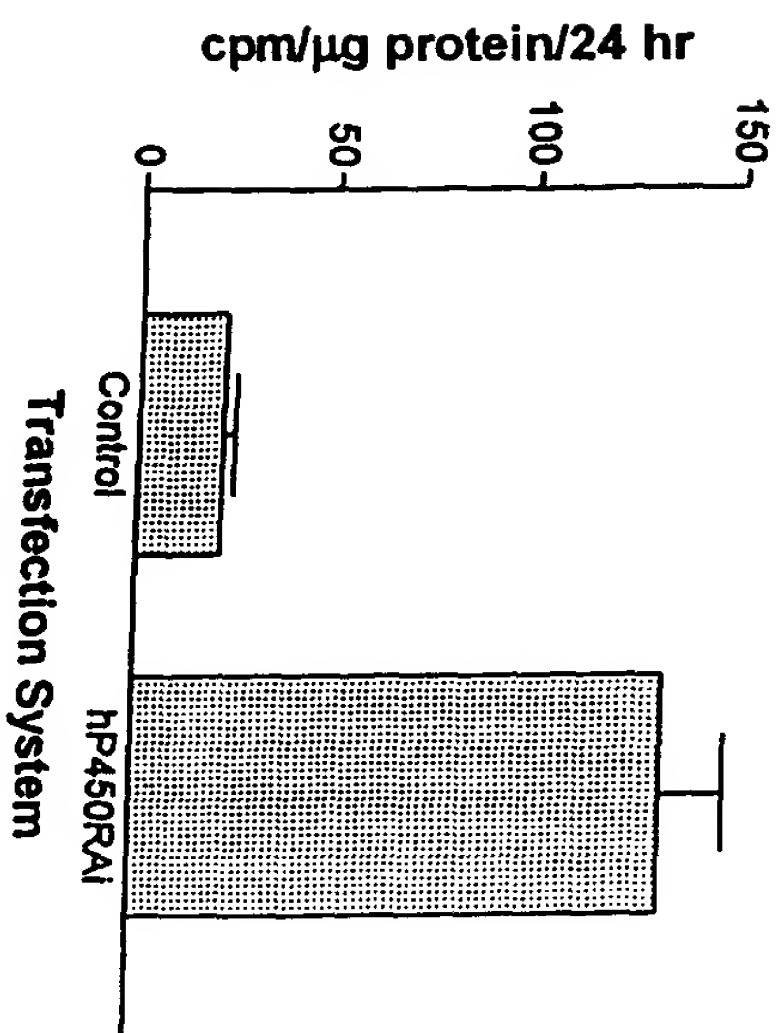


FIG. 11(a)

4-OH-RA Production in Transfected COS-1 cells

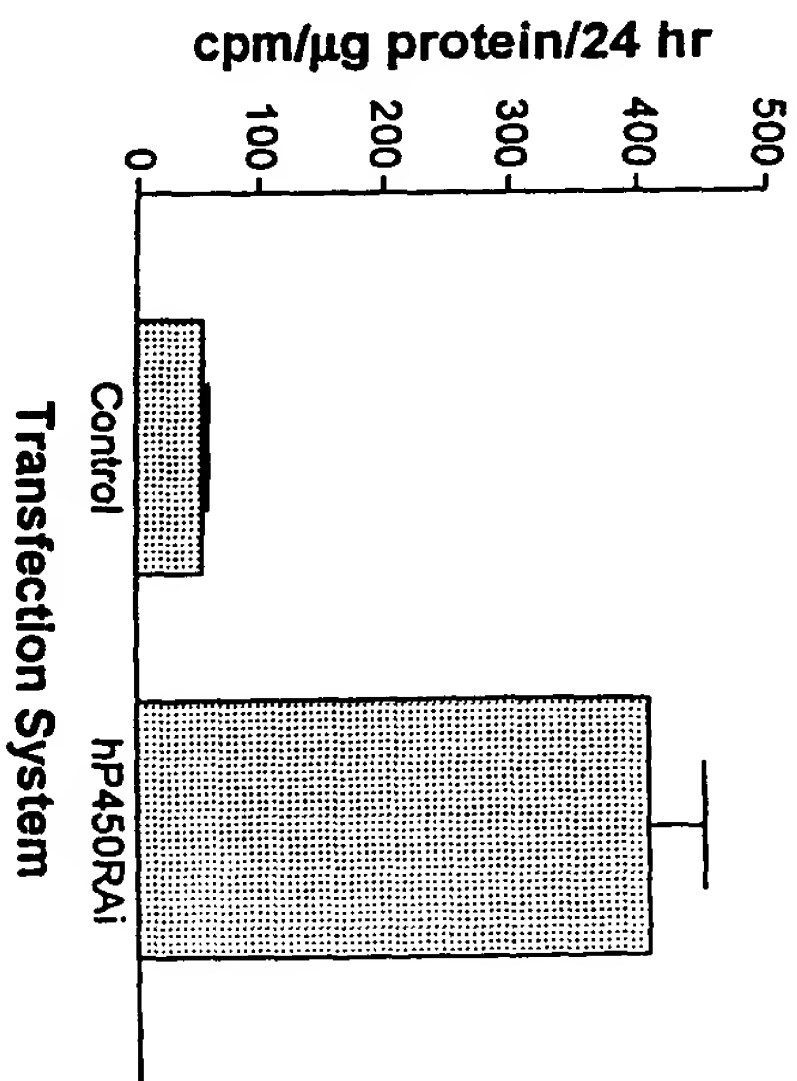


FIG. 11(b)

Formation of Aqueous Soluble Metabolites in Transfected COS-1 Cells

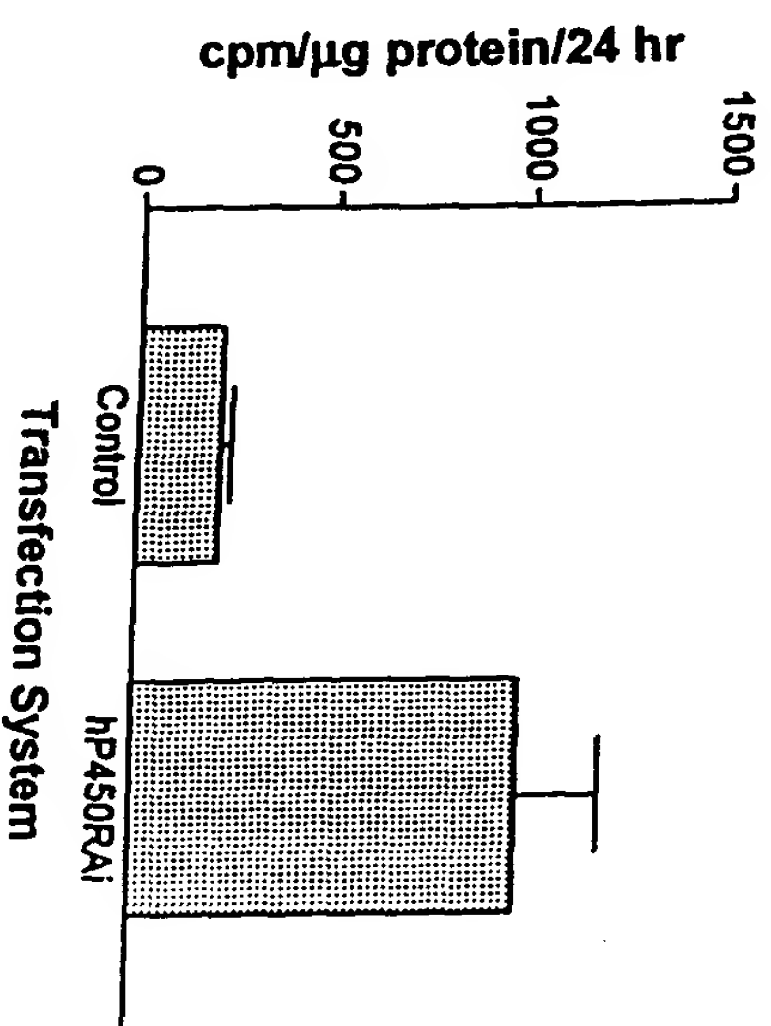


FIG. 11(c)

Unmetabolized RA in Transfected COS-1 Cells

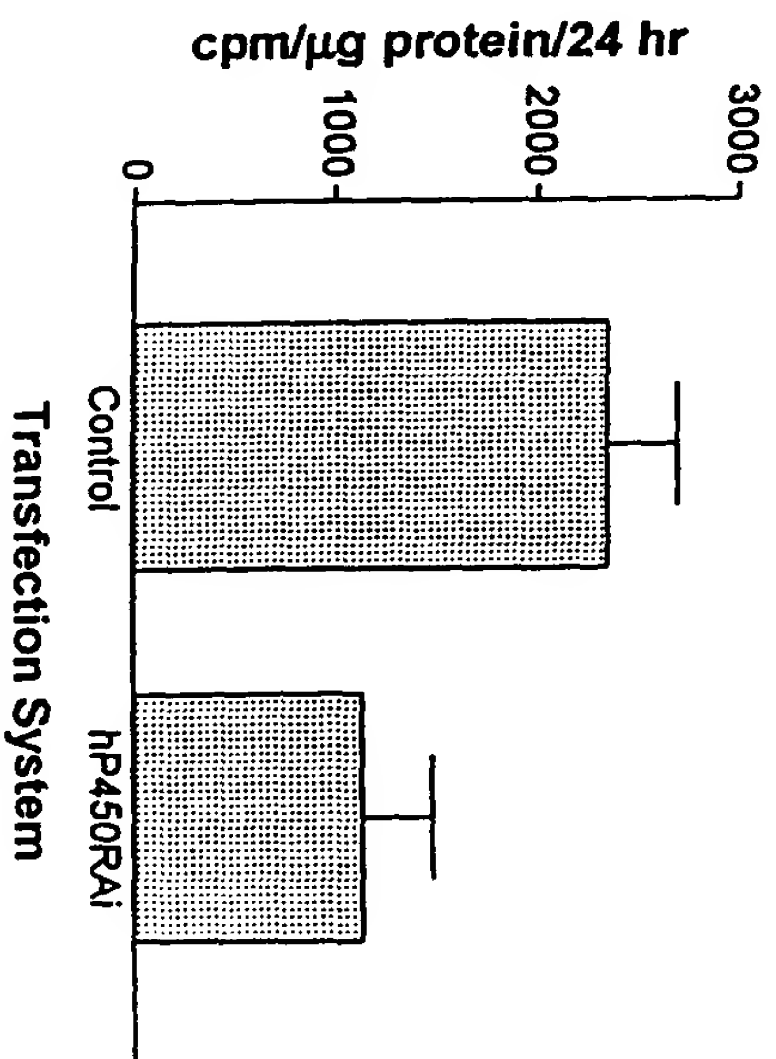
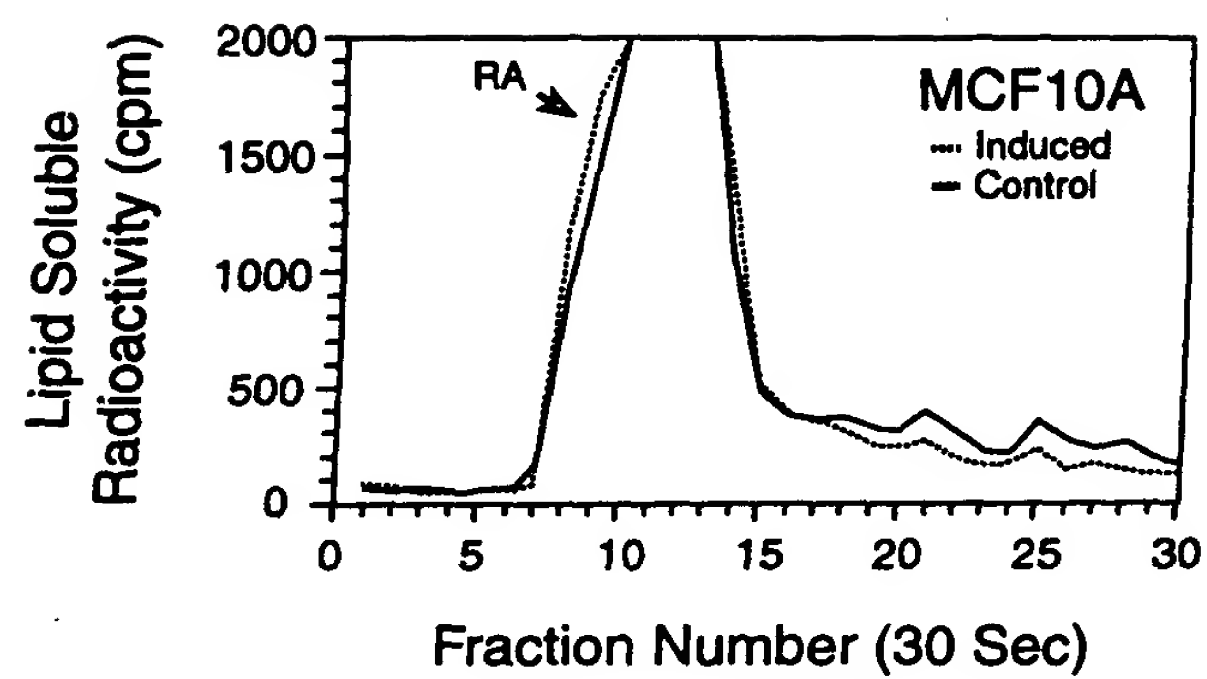


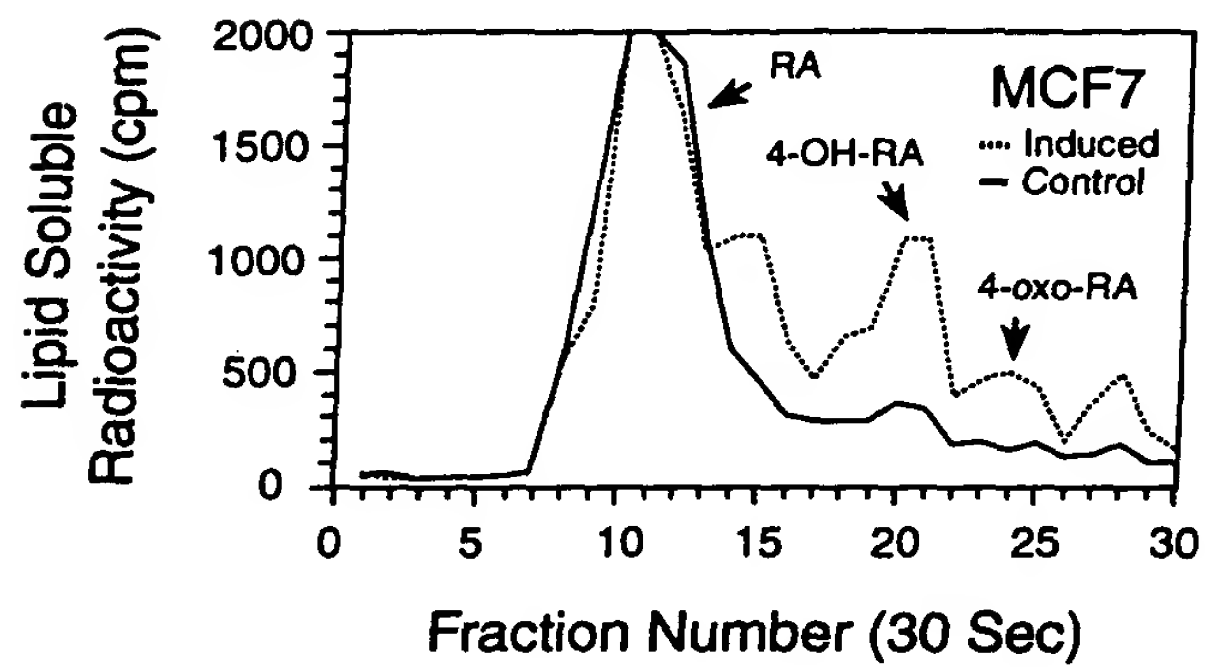
FIG. 11(d)

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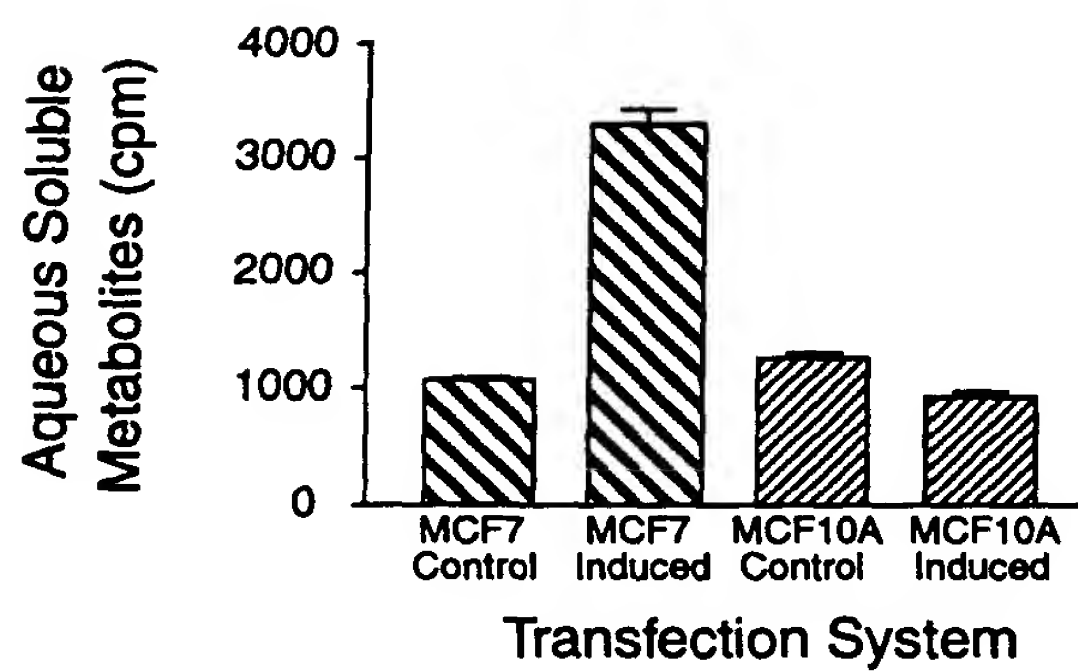
*a*



*b*



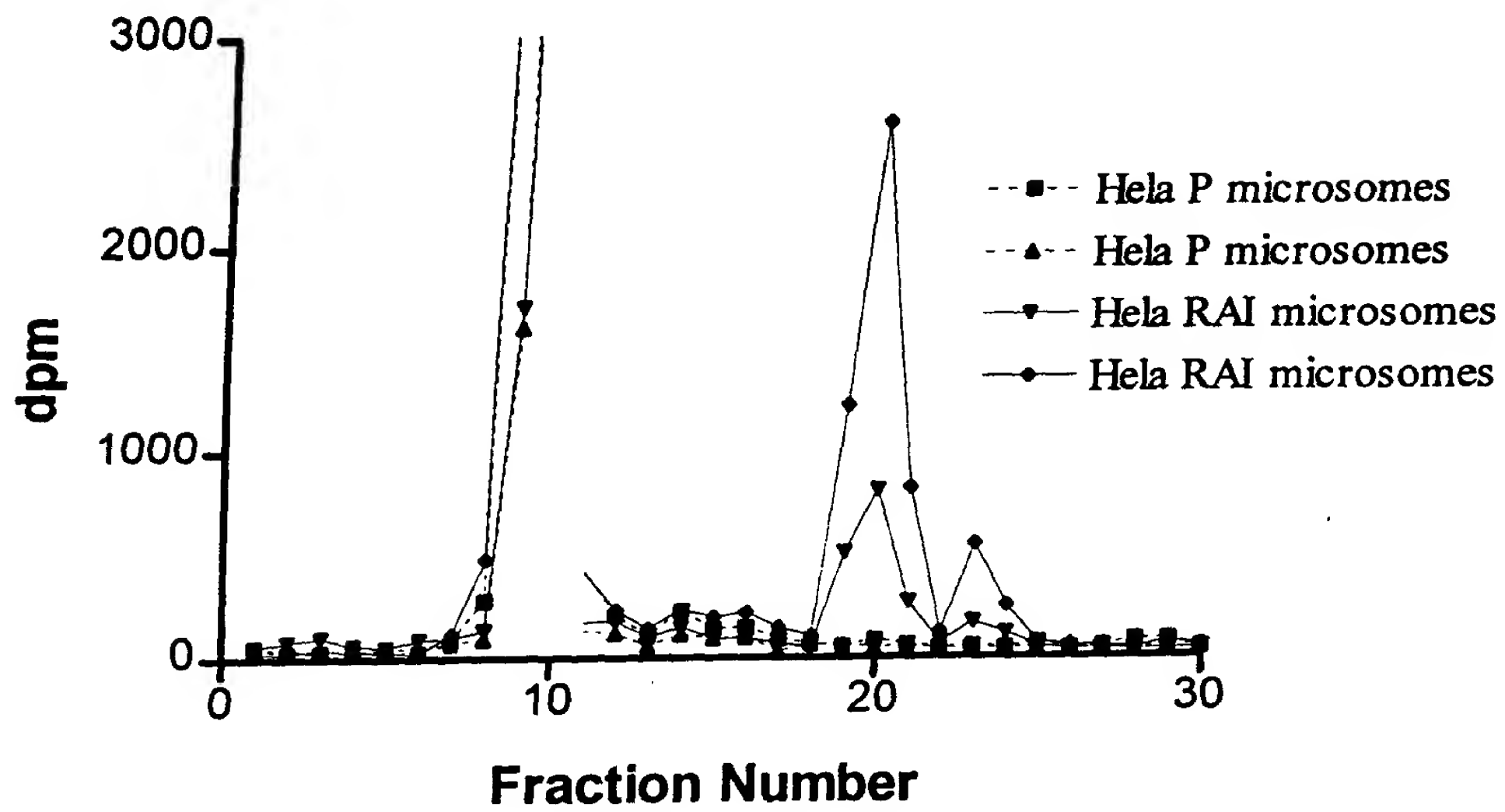
*c*



**FIG. 12**

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### Microsomal Preparations (90 minutes)



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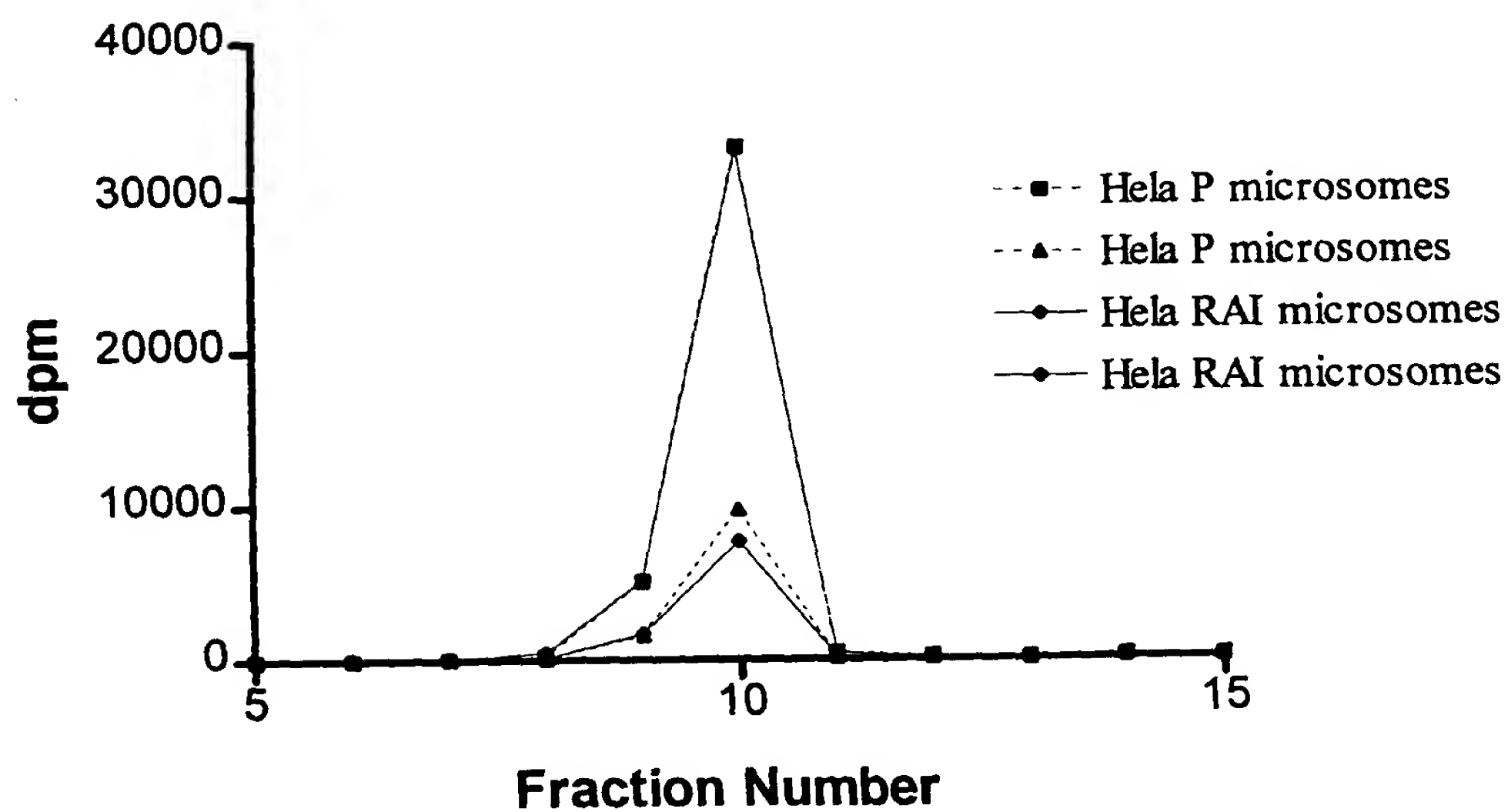


FIG. 13

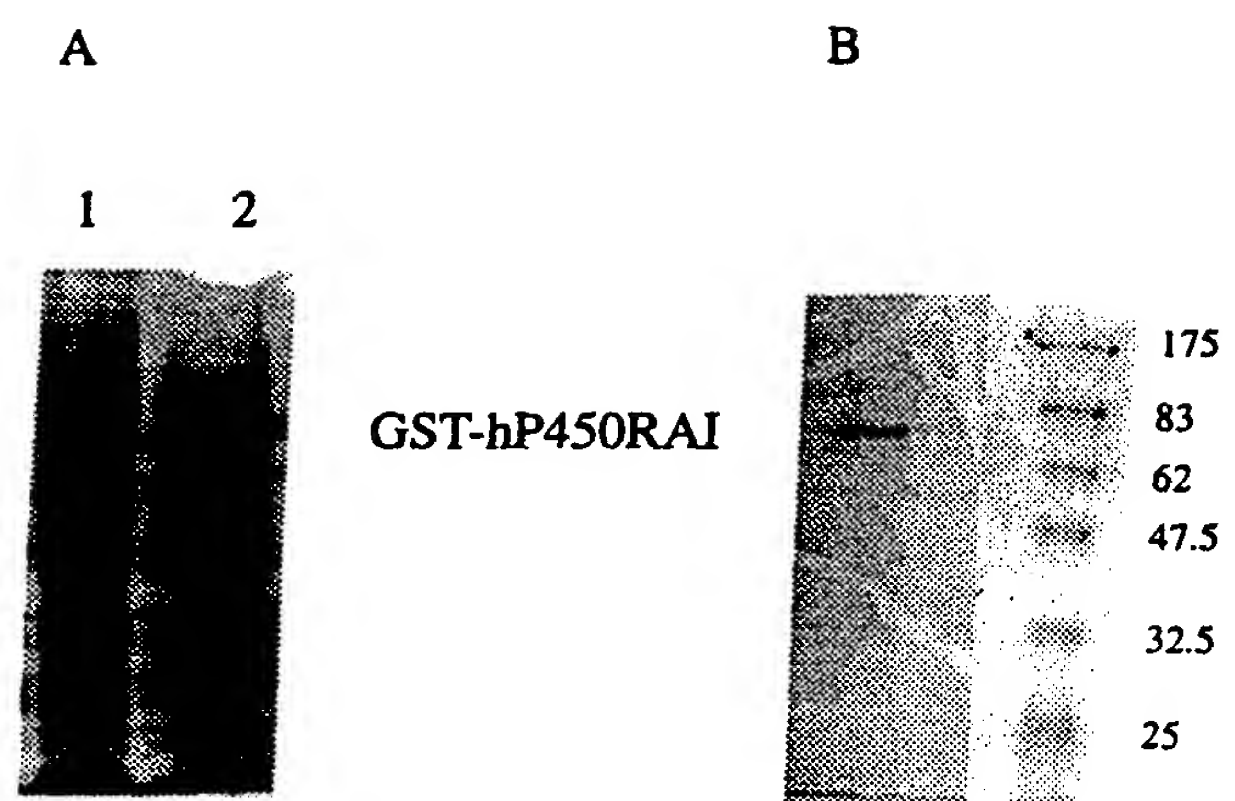


FIG. 14

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hRAI promoter -CG-----CA-C-----CC-----CAGGAGG  
 mRAI promoter -CG-----CACC-----CC-----CAGGAGG  
 zRAI promoter TCGGGGAATTAACACCTTTTCAAAGTGAAATCTCAGGATTGTCTGCCTTCTACAGGAGG

hRAI promoter -----CGCGCTCGGAGGGAA--GCCGCCAC--CGCCGCCGCTCTGCCTCGGCG  
 mRAI promoter -----CGCGCTCAGAGGGAA--GCCGCCAG--TG-CGCCGCTCTGCCTCGGCG  
 zRAI promoter TGGTATTAAATGCGCTATAACAAATGGTTGAGAGTTTGGAGCCGCTTCTGCCCTG---

hRAI promoter CGGAACAAACGGTTAAAGATTTT--GGGCCASCGCCTCCGCGGGGGAGGAGCCAGGGG  
 mRAI promoter CGGAACAAACGGTTAAAGATTTTTTTGGGC-AGCGCCT-CGAGGGGGAGGAGCCAGGGG  
 zRAI promoter -----TGGGC-----GGGGCGAG-----ATG--

hRAI promoter CCCCAATCCCGCAATTAAAGATGAACCTTTGGTGAACCTAATT-GTCTGACCAAGGTAACG  
 mRAI promoter CCCGA--TCCGCAATTAAAGATGAACCTTTGGTGAACCTAATTGTCTGACCAAGGTAACG  
 zRAI promoter -----ACACCACAATTAAAGATGAACCTTTGTGTGAACCTAATTATCTGAGGAAGTTAACA

hRAI promoter TGGGCAGCAACCTGGGCCGCC---TATAAGCGGCAGCGCCGTGGGGTTTGAAGCGCTG  
 mRAI promoter TGGGCAGTAACCTGGGCCGCC---TTATAAGAGGGCGCGCGGGGGTTTCGGAGCTAGG  
 zRAI promoter GGAGGAGACCTGCGCGCAATGGATATATAAGGGCGCGCAGGCGAGGACGCCCTCAGTTTG

hRAI promoter GCGGCGGCGGCAGGTGGCGCGGGAGGTCG-----CG  
 mRAI promoter GCGGCGGCGGCAGGTGGCGCGGGAGGCTG-----AA  
 zRAI promoter TGCGTAAAGACGCGTCTCCTCTCCAGAAGCTTGTTTTCGTTTGGCGATCAGTTGCGCG

hRAI promoter GCGCGCCATGG  
 mRAI promoter GCGTGCCATGG  
 zRAI promoter CTTCAACATGG

FIG. 15

# mRAI promoter transfection 2

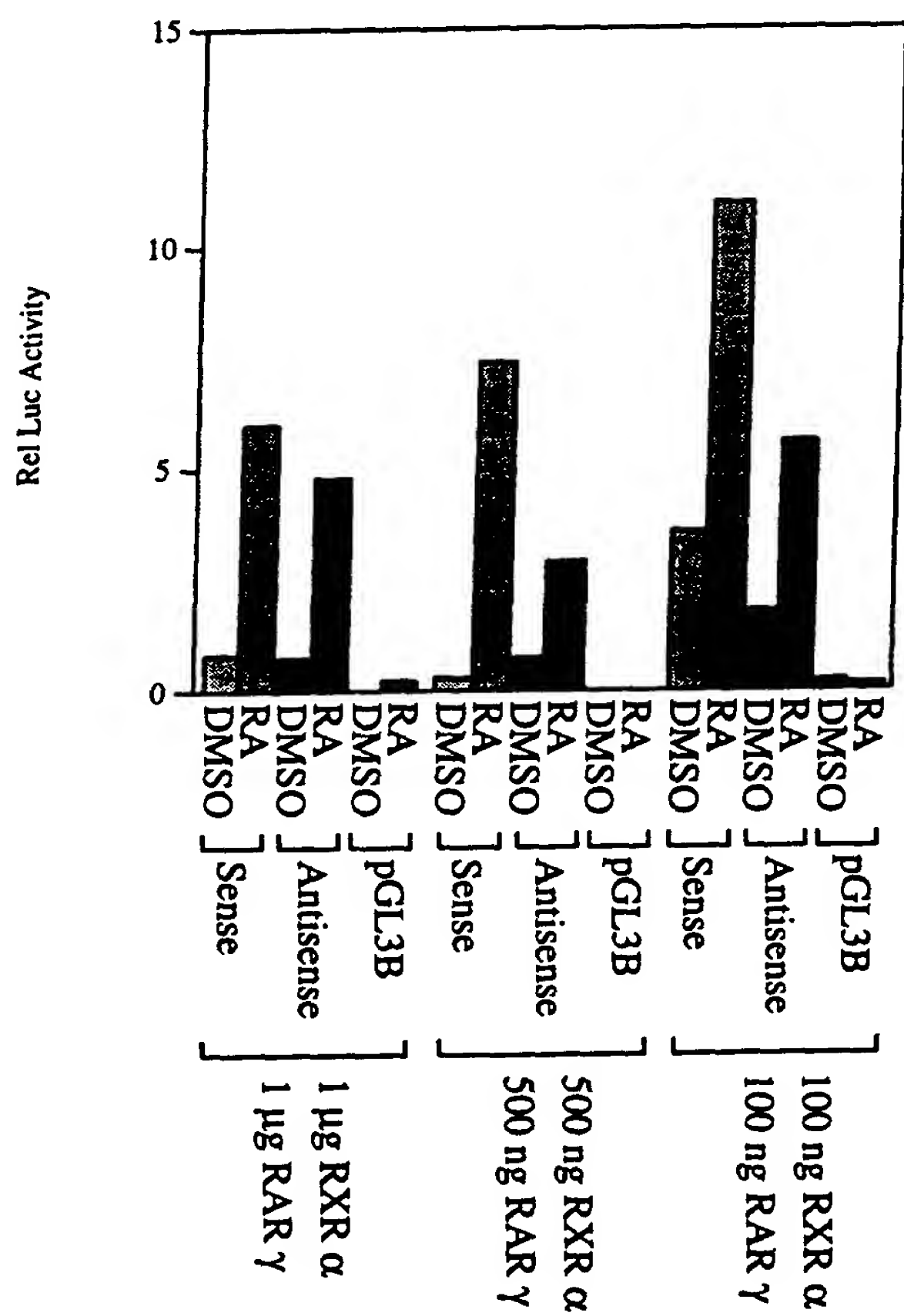


FIG. 16

09663432 092500



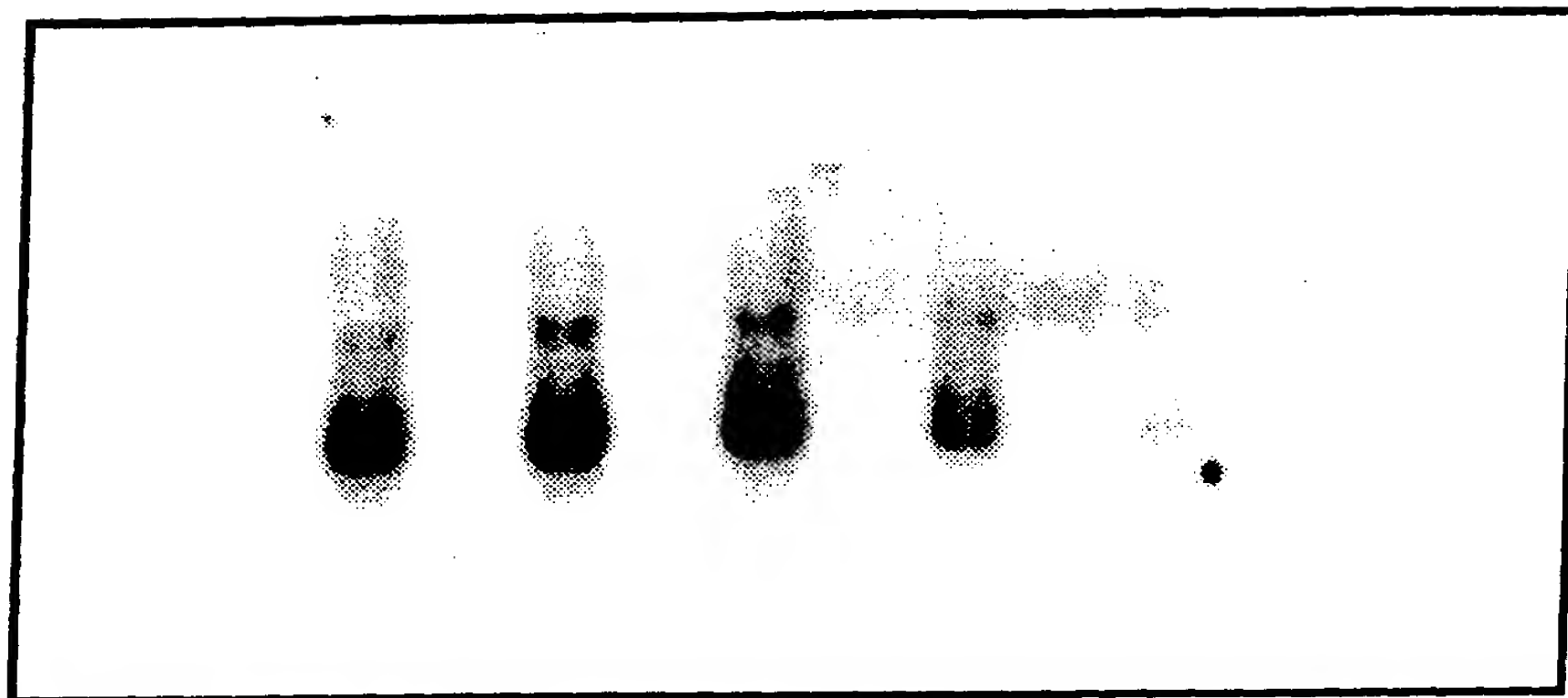
Cells were treated for twelve hours with the indicated concentrations of *all-trans* retinoic acid (*at*RA) and 4-HPR. Total RNA was extracted using TRIzol, and, following electrophoresis, Northern blotting was performed as described. The nitrocellulose was probed with radiolabelled P450RAI or GAPDH.

FIG. 17

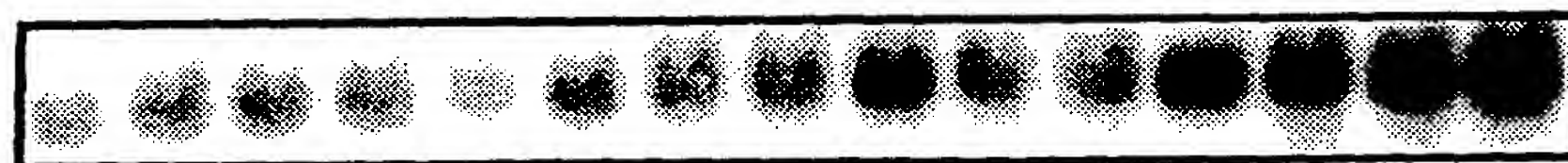
time (h)

0      1      3      6      12      24      36      48  
[+ -] [ + -] [ + -] [ + -] [ + -] [ + -] [ + -]

P450RAI



GAPDH



1    2    3    4    5    6    7    8    9    10    11    12    13    14    15

FIG. 18

095260 23489650  
095432 092500

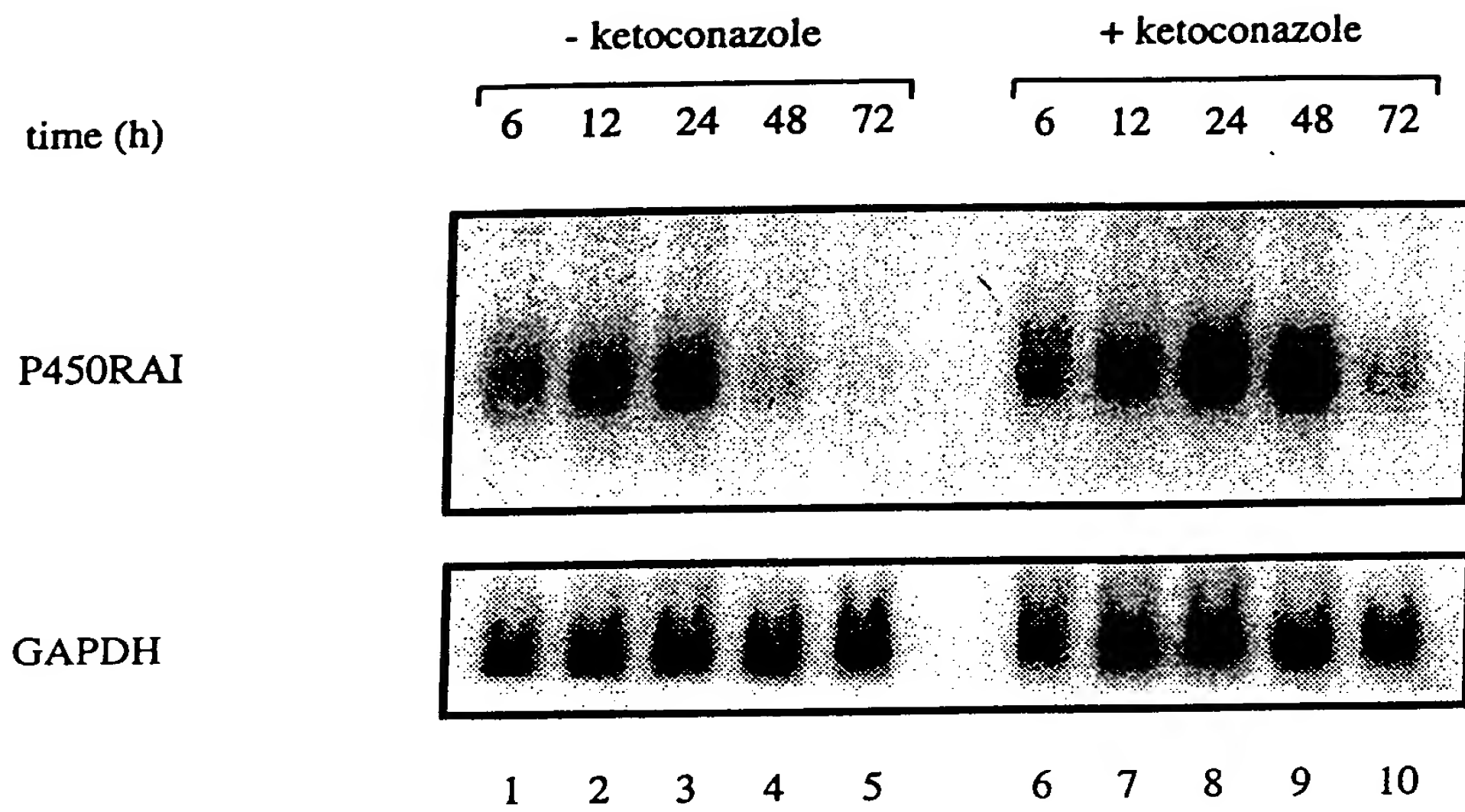


FIG. 19

005250" 23482950

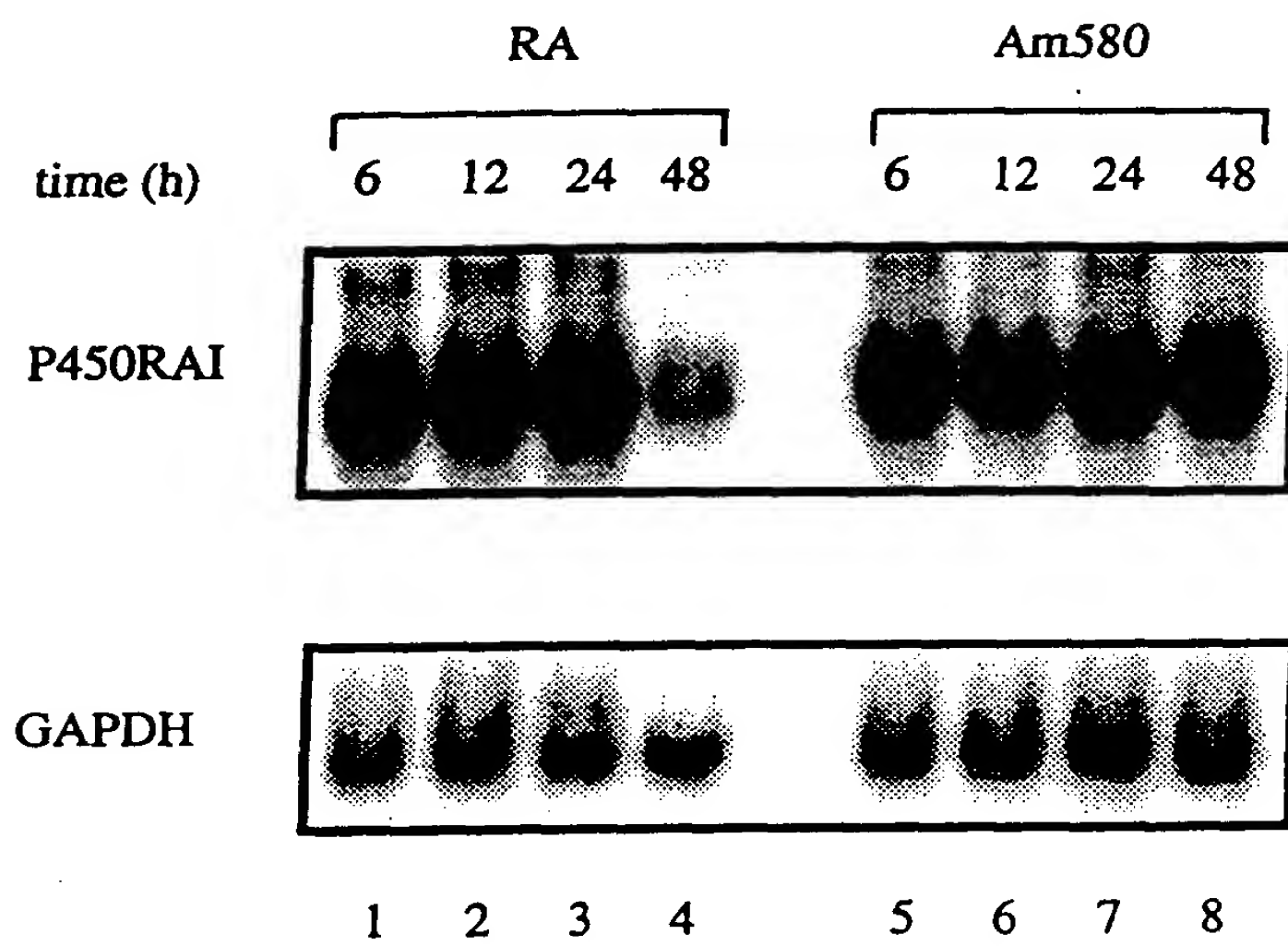


FIG. 20